

Use of a nucleic acid-binding chemotherapeutic agent, and a pharmaceutical composition

The present invention relates to a use of a nucleic acid-binding chemotherapeutic agent, wherein the nucleic acid-binding chemotherapeutic agent is capable of complexing a metal ion, yielding a complex that promotes the formation of hydroxyl radicals from hydrogen peroxide.

Such a nucleic acid-binding chemotherapeutic agent is already known in the art. For example, certain neoplastic tissues (tumours) may be treated with bleomycin. Bleomycin is capable of binding bivalent iron, while the ferro-ion retains its ability to promote the formation of hydroxyl radicals from hydrogen peroxide.

It is the object of the present invention to provide a novel use of a nucleic acid-binding chemotherapeutic agent such as defined above.

According to the present invention the nucleic acid-binding chemotherapeutic agent can be used for the preparation of a pharmaceutical composition for the treatment of a disease caused by virions.

Surprisingly it has been found that by applying the above-defined nucleic acid-binding chemotherapeutic agent, the virus replication may be inhibited, without visible detriment to the host cell. Without being bound to any theory, applicant believes that the inhibition is specific because the formation of hydroxyl radicals from hydrogen peroxide is promoted especially in virus-infected cells.

According to a preferred embodiment, the nucleic acid-binding chemotherapeutic agent is selected from the group comprising bleomycin, adriamycin, and their derivatives.

These compounds possess excellent metal ion-complexing properties. In particular, they are capable of binding ferro-ions in the body of a patient. This enables the ferroleomycin complex that is formed to promote the formation of hydroxyl radicals from hydrogen peroxide.

AMENDED SHEET

Preferably the nucleic acid-binding chemotherapeutic agent is used for the preparation of a pharmaceutical composition for the treatment of a disease caused by an RNA virus replication-inhibiting agent, in particular the nucleic acid-binding chemotherapeutic agent is used for the preparation of a pharmaceutical composition for the treatment of a disease caused by a HIV.

Carter, B.J. et al. (Proc. Natl. Acad. Sci. USA, volume 87, pp. 9373-9377 (1990)) describe the effect of Fe(II)-bleomycin complex on mRNA which codes for reverse transcriptase of HIV-1. The experiment described was performed in a cell-free system. There is no indication that the formation of hydroxyl radicals from hydrogen peroxide is promoted preferentially in infected cells.

The invention further relates to a pharmaceutical combination composition comprising a nucleic acid-binding chemotherapeutic agent comprising a metal ion complexed therewith, which complex is able to promote the formation of hydroxyl radicals from hydrogen peroxide, together with a pharmaceutically acceptable carrier or excipient, and which also comprises an iron-chelating compound which binds iron in a form in which it is unable to promote the formation of hydroxyl radicals from hydrogen peroxide.

Such an iron-chelator combination which optionally comprises two separate pharmaceutical compositions, each of which possessing one of the respective active components, facilitates more specific localization of the formation of the hydroxyl radicals. By using an iron-chelating compound that is unable to penetrate the cells, it is possible to preferentially prevent the formation of ferro-bleomycin complex outside the cells, and consequently also to reduce the damage that such a complex causes. At the same time, the use of an iron-chelating compound that is able to penetrate the cells, will limit the amount of ferro-ions that limit the formation of hydroxyl radicals. In this way at least part of the activation process of the transcription factor Nuclear Factor kappa B (NFkB), that can stimulate virus replication may be limited in the

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cytoplasm. However, it is necessary to ensure that iron is available for bleomycin. A physician may achieve this by choosing suitable doses of both active components, depending on the body weight of the person to be treated, and
5 the person's available iron level. According to a favour-

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able embodiment an iron-chelating compound is chosen having an iron-chelating capacity which is preferably at least three times lower, more preferably at least ten times lower than that of the nucleic acid-binding

5 chemotherapeutic agent.

Due to the greater affinity of bleomycin for iron, it is thus possible to promote the presence of active ferrobleomycin complex in infected cells and, in particular, to limit the extracellular detrimental effects of
10 bleomycin complex.

Applicant considers the possibility that the use of an iron-chelating compound as defined above may also be applied to limit undesired damage occurring during the treatment of neoplastic tissues with a nucleic acid-binding chemotherapeutic agent such as bleomycin.
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The present invention will now be exemplified by way of example and with reference to the drawing in which

Fig. 1 shows a graph representing the effect of bleomycin on the HIV-1 replication in macrophages;

20 Fig. 2 shows a graph representing the site of toxicity of bleomycin for macrophages;

Fig. 3 shows a graph representing the effect of bleomycin on the HIV-1 replication in lymphocytes;

25 Fig. 4 shows a graph representing the effect of the bleomycin concentration on the lymphocyte proliferation.

Example

Macrophages and lymphocytes (10^6 cells/ml) were infected with HIV-1_{Ba-L} for two hours. The ratio HIV particles/number of cells was 0.005 for macrophages and 0.001 for lymphocytes. The infected cells were then washed twice in order to remove excess virus. The cells were incubated for five days in RPMI 1640 medium (supplemented with 10% foetal calf serum, 10 U/ml of IL-2, 10 μ g/ml of gentamycin, and 0.5 μ g/ml of ciprofloxamine) with 3 iron chelators, being Deferoxamine (DI; Novartis Pharma, Arnhem, the Netherlands), Deferiprone (L1; Duchefa Farma B.V., Haarlem, the Netherlands) or Bleomycin (BLM; H. Lundbeck A/S, Copenhagen, Denmark). Virus in culture supernatant
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was inactivated with Empigen (Calbiochem-Novabiochem Co., La Jolla, California, United States of America) in a final concentration of 0.05% and subsequently heated for 30 minutes at 56°C. The p24 concentration was determined in an ELISA, as measure for the replication of HIV-1 (Moore, J.P. et al., Science 250, pp. 1139-1142 (1990)). Cytotoxicity measurements were carried out using a fluorescence-activated cell sorter with the aid of colouring with propidium iodide and DiOC5 (3,3'-diapentiloxacarboxyl amine iodide). The proliferation of lymphocytes was measured by incorporation of ³H-thymidine. Figure 1 and Figure 2 show the dose-dependent reduction of the HIV-1 replication. The limited cytotoxicity of bleomycin for macrophages is appears from Figure 3. The insignificant effect of bleomycin on the proliferation of lymphocytes is shown in Figure 4. In contrast with DF and L1 which do inhibit cell proliferation (results not shown; L1 inhibits the proliferation substantially completely at 10 µM), the cell proliferation with bleomycin remains intact over a wide concentration range; this fact indicates that another mechanism which is not based on the inhibition of proliferation, is involved. Likewise, the BLM-induced reduction of HIV replication is not a result of cytotoxic effects of BLM.

In an attempt to find out more about the level at which the nucleic acid-binding chemotherapeutic agent is activated to reduce the number of virions in an infected cell, the transcription factors present on HIV-LTR (HIV-Long Terminal Repeat) have been studied, of which NFκB plays an important role in viral transcription. For the initiation of the transcription of pro-viral DNA present in the host genome, it is necessary that NFκB is present. EMSE analysis (Electrophoretic Mobility Shift Assay) of NFκB in nuclear extracts showed that bleomycin has no effect on NFκB activation, suggesting that HIV inhibition due to bleomycin occurs along a path other than transcription inhibition. The fact that NFκB prepared from nuclear extract prepared from Jurkat cells stimulated with 20 ng/ml phorbol myristate acetate (PMA) were not inhibited by BLM (concentrations up to 3 µg/ml), suggests that the

inhibition of HIV-1 by BLM occurs in another manner than that proposed for conventional iron chelators such as DF (Sappey et al. Aids Res. Hum. Retroviruses 11, pp 1049-1061 (1995)).

5 In order to see whether bleomycin is active at an earlier stage, i.e. before integration into the genome, the viral DNA-damaging properties of BLM in peripheral blood lymphocytes (PBL) infected with HIV-1 were examined. To this end the products of reverse transcription, among
10 which was the first minus strand strong stop DNA, were amplified using the R/U5 primers: sense 5'-GGCTAACTAGGGAA-CCCACTG-3' and antisense 5'-CTGCTAGAGATTTTCCACACTGAC-3' (biotinylated at 5' end), which resulted in a fragment of 140 bp. To quantify this fragment, a digoxigenin-labelled
15 probe 5'-TGTGTGCCCCGTCTGTTGTGTG-3' was used. Quantification was carried out with the aid of a DIG detection ELISA (Boehringer-Mannheim, Mannheim, Germany). After incubation with BLM, strong stop DNA which was formed in peripheral blood lymphocytes (PBL) infected with HIV, was virtually
20 absent. This could either mean that the reverse transcriptase enzyme is inhibited, or that the DNA products of reverse transcriptase are damaged by BLM directly.

Based on experiments that have been carried out, it is believed that bleomycin damages viral DNA and/or RNA in
25 the cytoplasm. The GAPDH-DNA concentration in the cell measured as control (GAPDH stands for glyceraldehyde-3-phosphate dehydrogenase) remains substantially constant, supporting the idea that the host DNA is fairly well protected against BLM, and that BLM preferably attacks
30 DNA/RNA in the cytosol, in this case viral DNA/RNA. This could also explain why in the first experiment described above, the p24 values, after incubation of the cells with BLM, were not reduced completely. After all, as the cells are incubated in the absence of BLM for 2 hours, some pro-
35 viral integration into the host genome will undoubtedly have occurred.

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